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STRESS REDUCING EFFECTS OF OXYTOCIN IN A MATERNAL SEPARATION
PARADIGM

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

By

KEELY JANE O'CONNELL
B.S., Creighton University, 2006

2008
Wright State University

June 16, 2008

I HEREBY RECOMMEND THAT THE THESIS PREPARED
UNDER MY SUPERVISION BY Keely Jane O'Connell
ENTITLED Stress Reducing Effects of Oxytocin in
a Maternal Separation Paradigm BE ACCEPTED IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
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Abstract

This study compared the effects of centrally and peripherally administered oxytocin (OT) on HPA axis activity and the presence of both stress-induced active and passive behaviors in female guinea pig pups (*Cavia porcellus*) after 180-min of isolation . In Experiment 1, one dose of oxytocin (10µg/.2ml) was injected subcutaneously into the periphery. Plasma cortisol levels were reduced after 180-min of isolation and two passive behaviors, eye-closure and crouch, were reduced as well with the administration of oxytocin. In Experiment 2, two different doses (10µg/.2ml and 20µg/.2ml) were injected into the periphery with no significant results. In Experiment 3 one dose of oxytocin (1µg/5µl) was infused centrally. Plasma cortisol levels were marginally reduced after 30-min of isolation. Both the active and passive behaviors were unchanged.

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Introduction

Oxytocin is a neuropeptide hormone synthesized in the magnocellular neurons of the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus. One of the major peptides produced in the hypothalamus, this peptide is secreted by the posterior pituitary gland. Oxytocin has an extensive range of physiological effects produced through two separate systems: the peripheral and the central oxytocinergic systems (Ring, Malberg, Potestio, Ping, Boikess, Luo et al, 2006). The difference between the two is based on their distinct anatomy, functionality and sites of release and action (Ring, et al, 2006). Peripherally, oxytocin is best known for its hormonal role. In mammals, it is responsible for stimulating the smooth muscle of the uterus to initiate contractions during the birthing process and promoting lactation thereafter in the myoepithelial cells of the breast. Centrally, oxytocin can act as a neurotransmitter/neuromodulator and control some behavioral and physiological CNS parameters. This peptide is thought to influence various aspects of maternal (Numan & Insel, 2003), affiliative (Insel & Shapiro, 1992), and sexual behavior (Argiolas & Gessa, 1991). Physiologically, it may be able to suppress or initiate the release of various peptides also produced within the hypothalamus (Suh, Liu, Rasmussen, Gibbs, Steinberg & Yen, 1986).

Determining the influence of both central and peripheral oxytocin action is the blood brain barrier (BBB). This barrier's limited permeability discourages the passing of numerous substances such as many drugs and endogenous chemicals. Oxytocin, being non-steroid and water soluble, is carried freely within the blood; still, it is a peptide and therefore has limited ability to cross the BBB (~0.1% systemic oxytocin enters the brain; Jones & Robinson, 1982). However, there is evidence that suggests the BBB allows for

interaction between the two oxytocinergic systems. Research has shown that peripheral injections of oxytocin can mirror effects seen with central administration of this peptide. One such example is found in Mongolian gerbils where affiliation between monogamous females and their partners was increased by subcutaneous injections of oxytocin (Razzoli, Cushing, Carter & Valsecchi, 2003). With a short half life (only 4-5 minutes) and an unknown pathway of action in the periphery, oxytocin's prolonged effects remain unclear (Uvnäs-Moberg, 1998). Perhaps, it is crossing a weak area of the blood brain barrier (the circumventricular regions) in tiny, yet sufficient amounts or influencing the release or action of other compounds which, in turn, produce persistent CNS activity.

Central effects of oxytocin

In sheep, centrally administered oxytocin can significantly increase the frequency of most maternal behaviors (low-pitch bleats, sniffing, licking, and approaching/following the lamb; Kendrick, Keverne & Baldwin, 1987). Sheep are a useful model of bonding because unlike many other animals, ewes show highly selective maternal behavior only with their own lamb (Lim & Young, 2006). However, with vagino-cervical stimulation, a process that increases the release of oxytocin measured in cerebrospinal fluid and in the brain using microdialysis (Kendrick, Keverne, Baldwin & Sharman, 1986), acceptance of an unfamiliar lamb can be induced even following the ewe's bonding with her own lamb (Kendrick, Lévy & Keverne, 1991). The vagino-cervical stimulation mimics that of parturition, which appears to naturally cause the increase in oxytocin and subsequent acceptance of young. Oxytocin injected intracerebroventricularly (ICV) can, alone, induce acceptance of an unfamiliar lamb even

in non-pregnant ewes (Kendrick et al., 1987). Therefore, oxytocin plays a significant role in the bonding of the mother with her offspring.

One study demonstrated the role of oxytocin in partner preferences for both males and females in the monogamous prairie vole. Central administration of this peptide caused both males and females to exhibit increased social contact and significant preference for the familiar partner (Cho, DeVries, Williams & Carter, 1999). Though mating is typically required for formation of the monogamous pair bond, this bond forms even in the absence of mating with oxytocin administration (Cho et al., 1999; Williams, Carter & Insel, 1992). Further, formation of the pair bond has been shown to be blocked with the ICV infusion of oxytocin receptor antagonists even after extended mating bouts (Cho et al., 1999; Williams, Insel, Harbaugh & Carter, 1994; Winslow, Hastings, Carter, Harbaugh & Insel, 1993). This research provides clear evidence indicating that oxytocin is essential for social bonding under some conditions.

Possible reward role of oxytocin

Oxytocin has been found to play a key role in several prosocial behaviors. Examples include maternal behavior in sheep, affiliative behavior in several species such as sheep and rodents, and sexual behavior in prairie voles. Secretion of this peptide is thought to be induced through quiet physical contact with another animal (Carter, 1998). Oxytocin administration has been shown to inhibit the rise in circulating glucocorticoid levels; much like the presence of an attachment figure is able to do (Windle, Shanks, Lightman & Ingram, 1997). Therefore, with rodents, it has been hypothesized that maternal presence or contact induces oxytocin release in the young and, in turn, is rewarding to the pup (Nelson & Panksepp, 1996). In that study, infant rats that received

central administration of oxytocin tended to spend less time in contact with or near, their mother. The authors suggest that stimulation of oxytocin receptors reduced the motivation for, and the maintenance of, social contact (Nelson & Panksepp, 1996). These findings suggest that oxytocin contributes to the rewarding properties of social interaction for the pup and may help account for why social interaction is extremely important for young. Rewarding effects of positive experiences can become conditioned to neutral cues, implying that maternal presence alone, without physical contact, might induce oxytocin release. Indeed, though oxytocin is most likely released endogenously in the pup during the infant-mother attachment process via somatosensory stimulation, its release also has been shown to be elicited by nonnoxious stimuli such as warm temperature and odors, stimuli that the mother typically provides (Uvnäs-Moberg, 1998). All of these stimuli are believed to be rewarding to the pup and promote the animal's attachment to its mother.

Oxytocin and stress-related effects

Oxytocin appears to play a key role in reducing stress. Of particular relevance here, oxytocin has been found to reduce the activity of the hypothalamic-pituitary-adrenal (HPA) axis and behavioral signs of anxiety. The HPA axis plays a critical role in the body's response to stress. Its state of activation is often taken to indicate whether the body is experiencing some degree of stress. When activated by stressors, the PVN of the hypothalamus releases corticotropin releasing factor (CRF) which in turn activates the pituitary gland to release adrenocorticotropin hormone (ACTH). ACTH is transported to the adrenal gland where it initiates the release of glucocorticoids (primarily cortisol or corticosterone depending on the species) from the adrenal cortex. Cortisol enhances the

physiological response to stress, be it physical (e.g. electric shock, tissue damage) or psychological (uncertainty, novelty, or separation from attachment figure). The time course of plasma cortisol elevation varies greatly in length depending on the severity and duration of the stressor(s). Return of HPA activity to basal levels is facilitated by negative feedback mechanisms by which cortisol acts at central sites and the pituitary to inhibit further release of CRF and ACTH.

Central neuropeptides, including oxytocin, have been implicated in the control of the HPA axis. Prairie voles, when treated with oxytocin both peripherally and centrally, show reduced HPA activity (Carter, 1998). Experimental studies in other animals have shown that ICV oxytocin inhibits the stress-induced activation of the HPA axis (Neumann, Krömer, Toschi & Ebner, 2000; Uvnäs-Moberg, Ahlenius, Hillegaart & Alster, 1994; Uvnäs-Moberg, Bjökstrand, Hillegaart & Ahlenius, 1999; Windle et al, 1997). This also appears to occur in humans: in lactating women, a suppression of HPA activity has been observed if breast-feeding starts 30-60 min before exposure to a stressor (Altemus, Deuster, Galliven, Carter & Gold, 1995; Altemus, Redwine, Leong, Frye, Porges & Carter, 2001; Heinrichs, Meinlschmidt, Neumann, Wagner, Kirschbaum, Ehlert et al., 2001; Heinrichs, Neumann & Ehlert, 2002). Oxytocin's ability to reduce HPA activity may be due to the presence of both oxytocin and CRF containing neurons in the PVN. Oxytocin, somehow, either by blocking receptors or the actual production of CRF inhibits the release of CRF from the PVN, which, in turn suppresses HPA activity (Suh et al, 1986).

Oxytocin can produce anxiolytic-like effects; central and peripheral administration of oxytocin has been shown to reduce anxiety-like behavior in female rats

and mice (Bale, Davis, Auger, Dorsa & McCarthy, 2001; McCarthy, McDonald, Brooks & Goldman, 1996; Windle et al, 1997) Intense calling behavior is considered a measure of anxiety in the young of some species. The excessive calling behavior of isolated domestic chicks has been shown to be diminished following injection of oxytocin (Panksepp, Nelson & Bekkedal, 1997). In rat pups, separation cries were inhibited by central treatment with oxytocin (Insel & Winslow, 1991; Winslow & Insel, 1993). These examples suggest that oxytocin has anxiety reducing effects by way of both central and peripheral administration.

Male mice that were exposed to a pharmacologically validated preclinical model of anxiety, the four-plate test (FPT), were found to display behavior that indicated the anxiolytic-like effect of oxytocin as well. The four-plate apparatus consists of a Plexiglas chamber with a floor made of four identical metal plates. The plates are separated by a small gap (4mm) and electric shocks can be delivered through the metal plates. Foot shocks were delivered to the mice to suppress their exploration. If the mouse crossed from plate to plate despite the shocks given with each crossing, the number of shocks administered were recorded as “punished crossings”. Both peripheral and central administration of oxytocin produced dose-dependent increases in punished crossings (Ring, et al 2006). This particular experiment explored the anxiolytic-like effects of oxytocin, but perhaps, the oxytocin was also having an analgesic or antinociceptive effect causing the shock to be less painful or disruptive.

Oxytocin is also known for its anxiolytic-like effects in sexual intercourse. Thirty minutes after intercourse, male rats typically show increased time in the open arms of an elevated plus maze. Increased open-arm exploration (percentage of time spent on, and

number of entries into, open arms) indicates reduced anxiety. Central administration of an oxytocin receptor antagonist immediately after mating reduced the open-arm exploratory behavior of the male rats. Administration of this antagonist had no effect on anxiety-related behavior in nonmated rats, suggesting that an up-regulation of the oxytocin-system is responsible for the anxiolytic effect of mating (Waldherr & Neumann, 2007).

Anti-inflammatory properties of oxytocin

Oxytocin has antisecretory and antiulcer effects, facilitates wound healing and is involved in immune and inflammatory processes (İşeri, Gedik, Erzik, Uslu, Arbak et al, 2008). It can act as an anti-inflammatory and may also produce antinociceptive effects due to its anti-inflammatory properties. These properties have been shown to be comparable to dexamethasone, a powerful anti-inflammatory (Petersson, Wiberg, Lundberg & Uvnäs-Moberg, 2001). Some of the anti-inflammatory effects of oxytocin have been reported to be mediated through the inhibition of neutrophil-dependent oxidative damage (İşeri, Sener, Sağlam, Gedik et al., 2005).

The latex of the *Calotropis procera* plant produces inflammation of the skin and mucous membranes. In one particular study (Padhy & Kumar, 2005), aqueous extracts of dried latex of *C. procera* or carrageenan were injected into the subplantar surface of a rat paw 30 minutes after the subcutaneous administration of oxytocin. The resulting hyperalgesic response and functional impairment were evaluated during a 24 hour period. Oxytocin produced a dose-dependent decrease at 1mg/kg in edema formation by the dried latex and carrageenan, with a 32% and 40% inhibition, respectively. At 0.1mg/kg there was no significant effect. Oxytocin was fairly effective in ameliorating inflammatory

hyperalgesia. Its anti-nociceptive effects persisted up to 24 hours after exposure to the irritant. A mobility test (assessment of the animal's locomotor ability) showed that oxytocin prevented impairment; the pup was able to move about with limited difficulty. In a stair climbing test (assessment of the pup's ability to climb up three stairs of increasing height), treatment of oxytocin produced improvement in the task with dried latex as well as carrageenan-induced inflammatory hyperalgesia. The effect of oxytocin was more pronounced in the case of carrageenan.

In rats, intraperitoneal oxytocin was found to improve renal function in tissue cultures. The rats were treated with oxytocin and later underwent a nephrectomy in which the kidneys were removed for tissue cultures. The oxytocin protected the renal tissue against ischemia/ reperfusion-induced oxidative damage through its anti-inflammatory action (Tuğtepe, Sener, Biyikli, Yüksel et al., 2007). Oxytocin provided a therapeutic benefit by reducing the release of inflammatory cytokines, such as IL-1 and IL-6.

Interim Summary

Oxytocin is a neuropeptide involved in prosocial behaviors. It is hypothesized to be secreted endogenously in young animals either in the presence of, or contact with, their mother. This peptide is also capable of reducing the activity of the HPA axis and has anxiolytic-like properties, which include reducing the calling of distressed infants. Its protective role as an anti-inflammatory may shield body tissues from damage. All of these effects are thought to be initiated through oxytocin's natural endogenous release.

Maternal Separation

Maternal separation can be an extremely stressful situation for the young of many species. If placed alone in a novel environment, infants of several species exhibit HPA

activation and distinct behavioral changes (Rosenblum & Plimpton, 1981; Hennessy et al., 2001). In most species maternal separation produces an immediate increase in active behaviors, especially vocalizing. In some species of primates this is followed by a passive stage characterized by reduced vocalizing and locomotor activity, together with reduced responsiveness and overall activity and the occurrence of slouched or hunched postures. Studies with primates are of particular interest because primates display a specific emotional attachment to the mother that appears to mimic human attachment (Harlow, 1958).

The Guinea Pig Model

The guinea pig is a highly social rodent that has been found to display evidence of standard criteria used to define attachment in primates and humans. These include: recognition of and preference for the rearing figure as well as distressful reaction upon involuntary separation (Carter & Marr, 1970; Hennessy & Ritchey, 1987; Herman & Panksepp, 1978). Many similarities have been documented in the response of guinea pig pups and species of primate infants to brief maternal separation (Hennessy, 2003). When isolated, guinea pig pups exhibit HPA activation which, as in some primates is accompanied by an initial active, and subsequent passive, stage of behavioral responsiveness.

The active behaviors, vocalization and locomotor activity, are typically displayed immediately following separation. The pups emit high-pitched vocalizations thought to be contact calls (Hennessy & Moorman, 1989). These vocalizations also appear to reflect a heightened anxiety-like state since studies have shown that administration of anxiolytic drugs selectively reduce this particular behavior (Molewijk, Hartog, Van der Poel, Mos &

Olivier, 1996). If the pup remains isolated in a novel environment for about an hour or more, active behavioral responses wane and the animal begins displaying a constellation of passive responses, notably a characteristic crouched stance, prolonged closure of the eyes, and extensive piloerection. These passive responses appear to be governed by pro-inflammatory factors, such as cytokines, acting in the CNS. Pups that were given lipopolysacchride (LPS), a promoter of inflammatory responses, displayed the same constellation of passive behavioral effects as prolonged isolation from the mother typically elicits (Hennessy et al, 2004). Further, these specific behavioral responses have been shown to be reduced with anti-inflammatory administration (Schiml-Webb, Deak, Greenlee, Maken, & Hennessy, 2006). These passive responses appear specific to the absence of the mother because placing the pup into the test cage with the mother fully blocks these responses, whereas the presence of an unfamiliar adult female did not (Hennessy & Morris, 2005).

Hypotheses

The presence of the mother may be able to cause the release of oxytocin in her pups, which in turn may have stress-reducing effects on her young. These might include an ability to reduce HPA activity as well as, active (anxiogenic) and passive (pro-inflammatory mediated) behaviors. To test this hypothesis, we administered oxytocin to female guinea pig pups isolated from the mother. Only females were used in this study because oxytocin often has a greater impact on females than it does on males (Cho et al, 1999). In this exploratory study, oxytocin was administered to pups both peripherally (via injection) and centrally (via ICV infusion) because both routes of administration

were effective in previous studies. Isolation behavior was monitored and blood samples were taken for analysis of HPA activity via cortisol assays.

General methods

Subjects

Female albino guinea pigs (*Cavia porcellus*) of the Hartley strain were bred in our laboratory and housed with their mothers and littermates. They remained in plastic cages (75cm x 54cm x 24cm) filled with sawdust bedding located in our laboratory colony room. Their diets consisted of water and guinea pig chow which were always freely available. The lights in the colony room were maintained on a 12:12 light/dark cycle with lights on at 7:00 am and out at 7:00 pm. The testing rooms and colony rooms were maintained between 65 and 70 ° F. All of the procedures used were approved by the Wright State University Laboratory Animal Care and Use Committee.

ICV surgery

Pups (≥ 190 g) underwent surgery for placement of a cannula aimed at the right lateral ventricle between days 16 and 19 under isoflurane anesthesia with additional local anesthesia to the scalp (0.25mg/0.1ml 0.25% bupivacaine). Guide cannulae (26 gauge) were placed relative to bregma with coordinates of -3.0mm anterior-posterior, -3.0 medial-lateral, and -4.0mm dorsal-ventral, and a stainless steel screw was placed adjacent to the guide cannulae to help secure the cranioplastic cement. All cannula supplies were sterile at the time of surgery and were purchased from Plastics One (Roanoke, VA). All pups were treated with buprenorphine (0.015mg/0.05ml) post-surgery to control for pain. Following surgery animals were weighed daily and dummy cannulae were checked for

patency. Animals were allowed to recover from surgery for at least 4 days prior to the first test.

Drug Administration

Each pup was tested twice, once with a vehicle solution and once with a particular dose of oxytocin (Bachem Laboratories). The oxytocin doses were prepared in our laboratory and both the oxytocin and the vehicle solution were kept at -80° C until just prior to injection or central infusion. Typically, no more than one pup per litter was used in an experimental group; however, there were a few exceptions in Experiment 2 where more than one pup per litter received the same dose of oxytocin. For peripheral administration, pups were removed from their home cages and injected subcutaneously in the nape of the neck with oxytocin or saline. Once injected, the pup was returned to the home cage for 20 minutes prior to testing. For central administration, pups were removed from their home cages and infused (1µl/ 30 seconds) with 5µl of artificial cerebrospinal fluid (aCSF) vehicle, or oxytocin in aCSF vehicle with a Hamilton microsyringe. The tip remained in place for an additional 30 seconds to ensure all the fluid was administered. Once infused, the pup was returned to the home cage for 20 minutes prior to testing.

Test Procedures

For testing, the pup was taken in a carrying cage to the test room and placed in a transparent, plastic testing cage (47cm x 24cm x 20cm) with a clear vented lid. The test room was kept approximately at 67°F. If the testing room is found to be below 65°F, a space heater was used to adjust the temperature before testing began.

A pup's first test took place between days 19-22 (with day 0 being the day of birth), and the second between days 23-26 with a minimum of three days between

behavior tests. The order in which oxytocin and the vehicle were administered was counterbalanced across pups. Pups were exposed alone to the test cage for three hours, with behavior observed at 0-30, 60-90, and 150-180 minutes. The pup's two tests always started at the same time of day; 10:00 am-1:00 pm, 10:30 am-1:30 pm, 12:00 pm-3:00 pm, 12:30 pm-3:30 pm, or 4:00 pm-7:00 pm.

Behavioral observation

Active and passive separation behaviors were scored by a trained observer through one way glass. The behaviors noted, with pencil and paper, included: vocalizations, line crossings, crouch, eye closure, and piloerection. A microphone situated above the test cage was used to transmit vocalizations to a headphone worn by the observer. "Whistle" vocalizations were tallied on a hand counter. Locomotor activity was measured by the number of times the pup crossed any of four lines dividing the testing cage into four equal sections. Whereas vocalizing and line-crossing (the active behaviors) were scored continuously, one-zero sampling was used for the passive responses (crouch, eye closure, piloerection), which tended to occur over prolonged periods of time. The number of 60-s intervals in which the animal displayed the crouched stance (body hunched down with head lowered and feet tucked beneath), eye-closing (more than 1 s of sustained complete closure or near-complete closure of one eye or both eyes), and extensive piloerection (occurring over most of the visible body surface) was scored (i.e., maximum score = 30 for each 30-min observation session). When all three of these passive behaviors are seen simultaneously, they are termed a "full passive response".

Blood sampling and cortisol determination

Ear veins located along the periphery of the external ear were used to collect blood for cortisol analysis. One experimenter held the pup on his or her lap while a second experimenter pricked the blood vessels with a 23g injection needle. The blood was collected in a capillary tube as the ear was massaged to increase blood flow.

During the 3- hour separation period, blood samples were taken after 30 minutes, 90 minutes, and 180 minutes. Blood samples for estimates of resting levels were collected at the same time of day at least 24 hours after the first, and 24 hours before the second, isolation tests. The blood samples were collected within 5 minutes of the initiation of disturbance. This is rapidly enough to ensure cortisol levels in the samples obtained were not affected by its sampling procedure (Sachser, 1994). The blood was centrifuged and the plasma was separated and frozen until the time of assay. Cortisol concentrations were determined in duplicate using a standard radioimmunoassay kit (¹²⁵I ‘Coat-a-Count’ Cortisol, Diagnostic Products, Los Angeles, CA). Intra- and inter- assay coefficients of variation were 8.9% and 3.2%, respectively.

Data Analyses

Because of large numbers of zero scores and otherwise nonnormal distributions of nearly all measures, and for consistency and ease of presentation, nonparametric tests were used for all behavior analyses, and central tendency and variability are represented by medians and semi-interquartile ranges. Cortisol levels were assessed with analysis of variance (ANOVA) or *t*-test, with central tendency and variability represented with means and standard errors. Because some animals did not provide complete data (primarily due to an inability to collect sufficient blood) sample sizes varied from 9 to 18 for different measures.

Experiment 1

To test the behavioral and physiological effects of peripheral injection of oxytocin in isolated guinea pig pups, oxytocin was injected subcutaneously.

Method

Each pup was tested once with a saline solution and once with a 10 μ g dose of oxytocin in a volume of .2 ml. This dose of oxytocin was chosen because it has been found to have behavioral effects in rodents of similar size to guinea pig pups (Witt, Carter & Walton, 1990).

Results

Cortisol. The repeated measures ANOVA showed a significant Drug X Time interaction, $F(2, 16) = 5.71$. Follow-up simple main effects tests revealed that subcutaneous injection of 10 μ g oxytocin significantly reduced the levels of plasma cortisol in isolated female guinea pig pups at 180 minutes compared to the vehicle saline, $p < 0.01$. There was no significant effect at either the 30 or 90 minute time points.

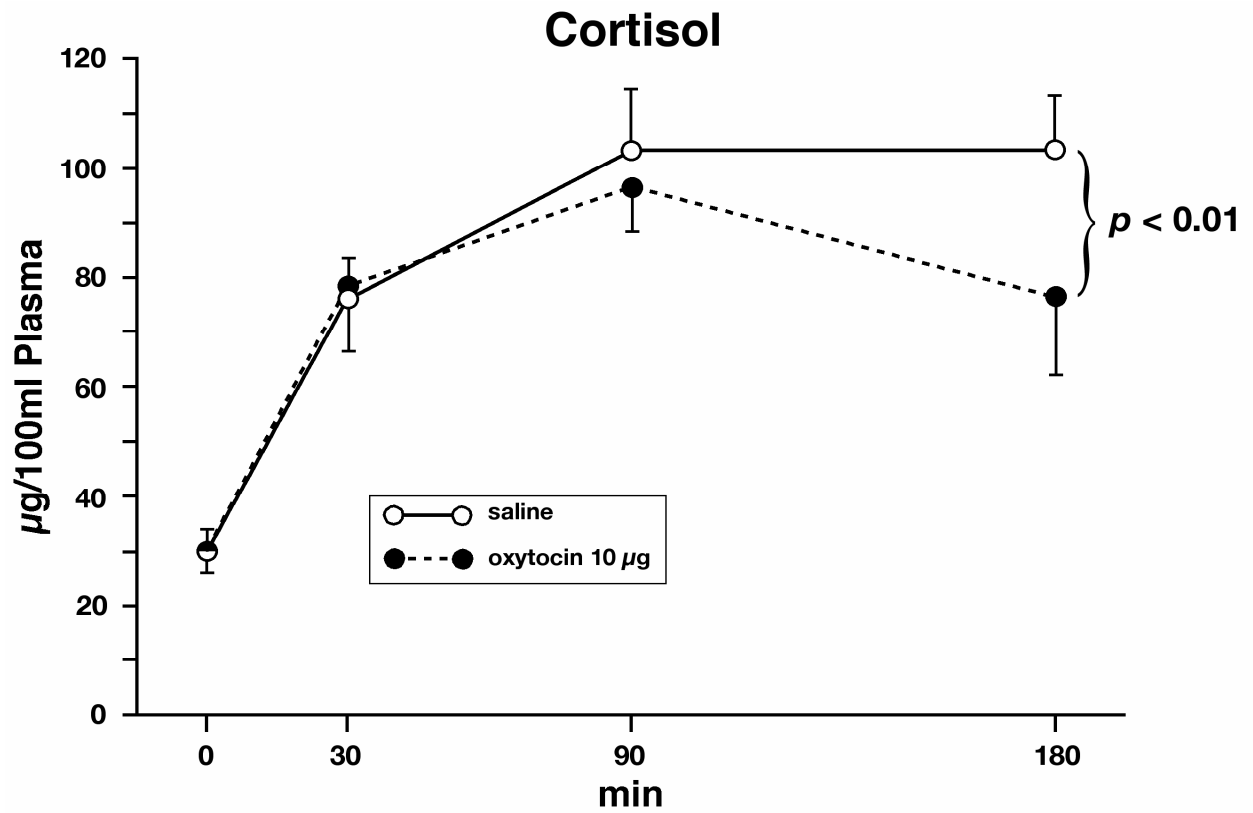


Fig.1. Mean plasma cortisol levels and standard errors (vertical lines) of female guinea pig pups ($N=9$) in the base (0 min) condition and 30, 90 and 180-min following exposure to the novel environment in the oxytocin and saline conditions.

Active Behaviors. The Wilcoxon test showed that the administration of oxytocin did not significantly affect the active behaviors (vocalization and line-crossing) in comparison to the vehicle in isolated guinea pig pups ($N=15$). The semi interquartile ranges (SIR) were greater than the median values for both groups, indicating a high degree of variability in scores.

Table 1

Median and Semi-Interquartile Range (SIR) of Active Behaviors in Experiment 1

	Oxytocin(10μg)	Saline
Vocalizations		
Median	50	258
SIR	664	443
Line-Crossings		
Median	5	5
SIR	9	23

Passive Behaviors. The Wilcoxon test showed that pups exhibited a marginally significant decrease in crouching ($p < 0.08$) and a significant reduction in eye closure ($p < 0.05$) with the administration of oxytocin compared to those given the vehicle, saline. No difference was found for piloerection or the full passive response.

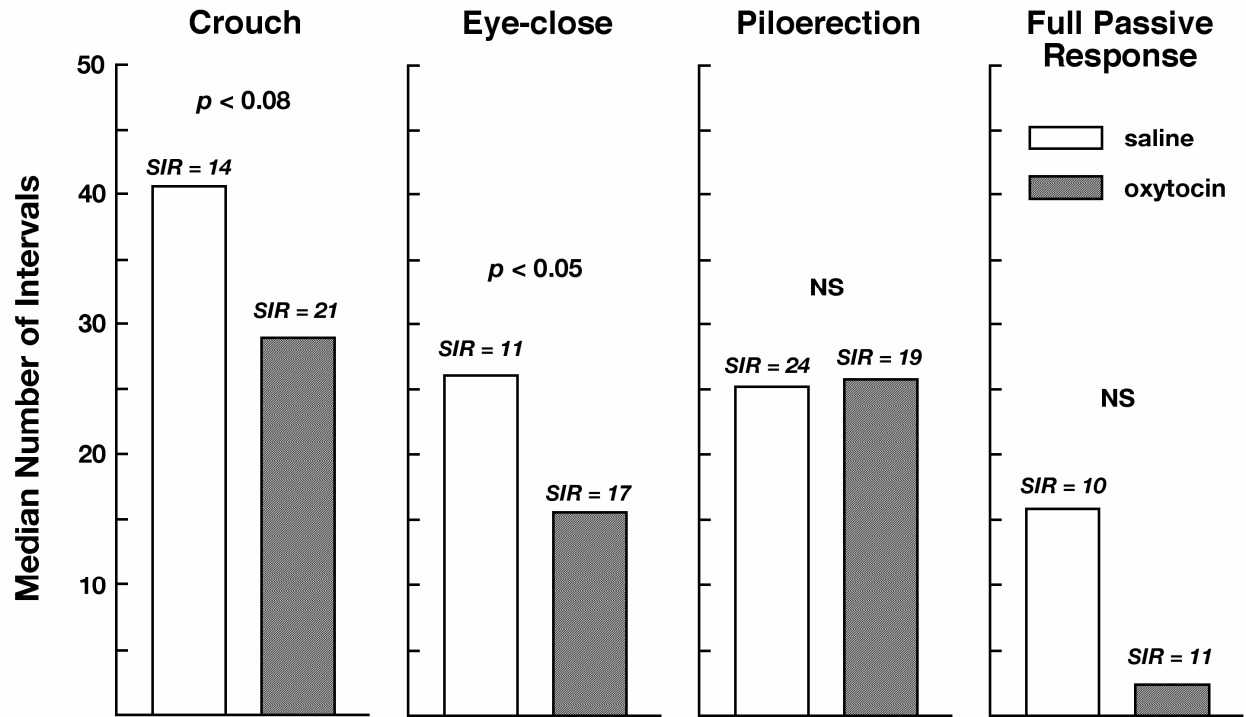


Fig.2. Median and semi interquartile range (SIR) number of crouch, eye-close, piloerection, and the full passive response observed in female guinea pig pups during 180-min isolation test in Experiment 1.

| *Discussion*

Oxytocin reduced plasma cortisol levels in isolated guinea pig pups at 180 minutes of separation. The active behaviors scored were not significantly affected. The vocalization values were numerically lower with administration of oxytocin as opposed to saline. However, the great variability of the scores was more striking. For example, of the 15 animals tested, the number of vocalizations documented ranged from 0 for some animals to thousands for others in the same condition. The passive behavior of eye closure was significantly reduced by oxytocin while crouch was marginally reduced. The other two behaviors were not significantly different. It appears then that the oxytocin administration procedure used here had a small moderating influence on the passive response.

Experiment 2

In Experiment 1, one of the four passive behaviors was significantly reduced and cortisol was found to be significantly lower at 180-min with the administration of oxytocin. Therefore, it was thought that increasing the dose might enhance the effects of oxytocin. Furthermore, because there was no sign of an effect of oxytocin on cortisol levels at 30 or 90-min, and because the disturbance of collecting these samples might have influenced behavior, Experiment 2 assessed cortisol responses only at the 180-min time point. Both a 10 and 20 μ g dose were administered.

Method

Two groups of pups, one receiving 10 μ g dose and the other a 20 μ g dose, were each tested twice, once with oxytocin and once with saline, with blood samples collected only at 180 minutes to reduce disturbance.

Results

Cortisol. A paired *t*-test of 180-min cortisol values showed that subcutaneous injection of either 10 µg (*N*= 18) or 20 µg (*N*=14) oxytocin had no measurable effect on the cortisol response.

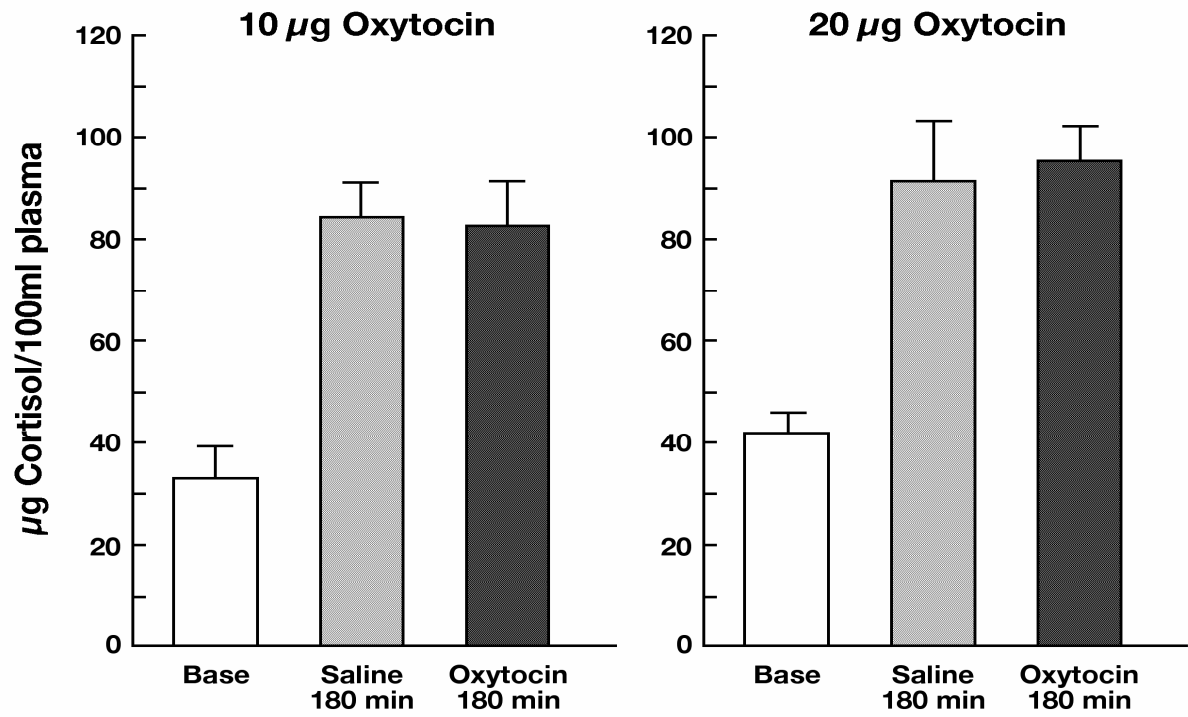


Fig.3. Mean resting plasma cortisol levels and levels at 180-min following oxytocin or saline injection in Experiment 2

Active Behaviors. The Wilcoxon test revealed no significant differences in the number of vocalizations or line-crossings exhibited following administration of 10 μ g ($N=18$) or 20 μ g oxytocin ($N=18$) vs. saline in female guinea pig pups. As in Experiment 1, the semi-interquartile ranges indicate high variability in the data.

Table 2

Median and Semi-Interquartile Range (SIR) of Active Behaviors in Experiment 2

10µg		Oxytocin	Saline
10µg	Vocalizations		
	Median	925	389
	SIR	797	671
	Line-crossings		
	Median	5	13
	SIR	29	12
20µg	Vocalizations		
	Median	330	573
	SIR	945	867
	Line-crossings		
	Median	10	3
	SIR	23	16

Passive Behaviors. The Wilcoxon test revealed no significant difference for crouch, eye-close, piloerection, or the full passive response with administration of either 10 μ g ($N=18$) or 20 μ g ($N=18$) oxytocin vs saline in guinea pig pups.

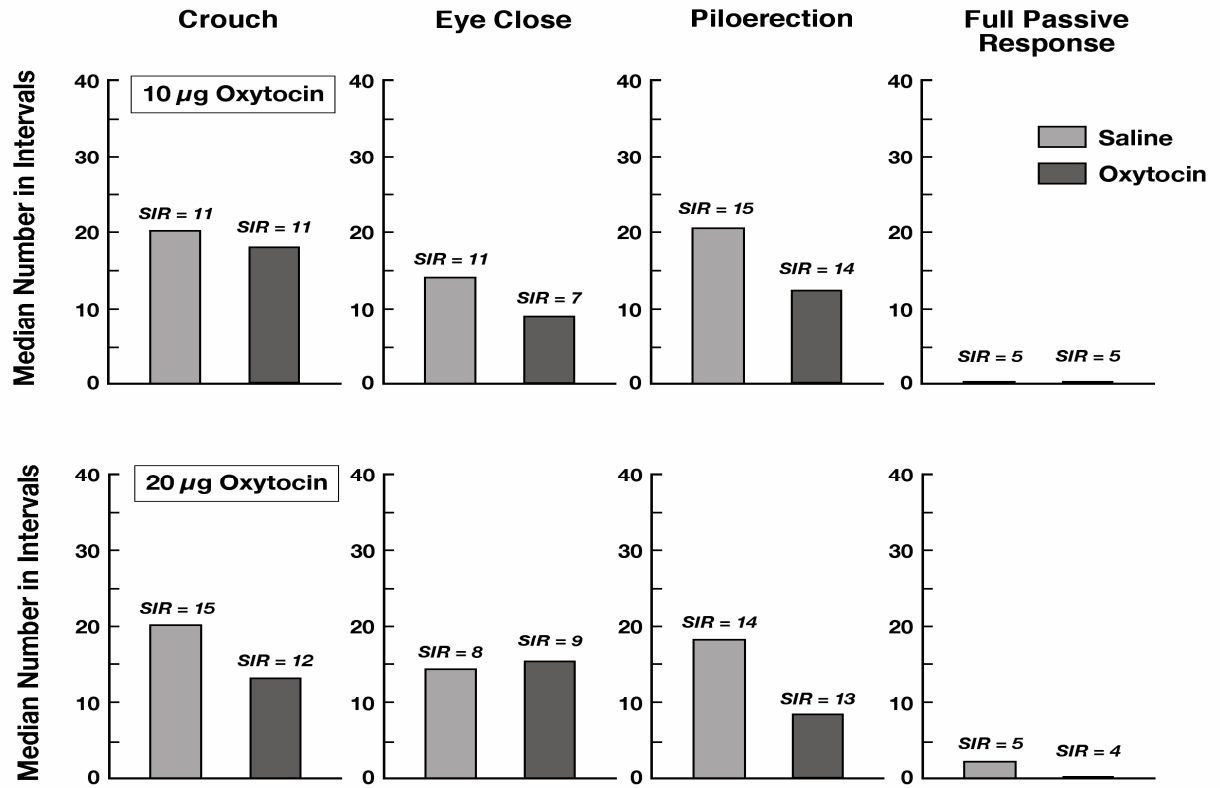


Fig.4. Median and semi interquartile range (SIR) number of crouch, eye-close, piloerection, and the full passive response observed in female guinea pig pups during 180-min isolation test in Experiment 2

Discussion

This experiment was quite similar to the first, but did not reveal any significant effects. The only differences between the two experiments were the addition of a second dose of oxytocin, 20 μ g, and the elimination of two blood samples (at 30 and 90-min). The first two blood samples were eliminated in Experiment 2 in order to reduce disturbance of the pup, which was thought to possibly reduce measurable effects of oxytocin. Instead the results suggest that the disturbance may have enhanced oxytocin's effects.

Experiment 3

In Experiments 1 and 2 oxytocin was administered via peripheral injection. In this experiment, oxytocin was administered centrally through an intracerebroventricular (ICV) cannula. Oxytocin has limited permeability through the blood brain barrier; therefore, central administration of oxytocin guarantees its presence within the brain.

Method

A 1 μ g dose of oxytocin was chosen because this is a commonly used dose in rodents of the approximate size of guinea pig pups (Cho, et al., 1999). Initially, blood samples were taken three times on the testing day: 30, 90 and 180-minutes following isolation. A preliminary analysis of subjects indicated that no differences were apparent at 90 minutes and so this sample was omitted in tests of later tested pups to minimize disturbance. There was no difference in the response of pups that did and did not have blood collected at 90-min, and so data for the two subgroups were combined. Following the last behavior test, all pups were euthanized and the correct placement of the cannula

was confirmed by injecting dye into the cannula. Only pups found to have dye in the lateral ventricle(s) were included in analyses.

Results

Cortisol. The repeated measures ANOVA displayed a marginally significant Drug X Time interaction, $F(1, 11) = 4.11, p < 0.07, N = 12$. A planned comparison of the 30 min value showed that oxytocin significantly reduced the levels of circulating cortisol in pups compared to those who received aCSF, $p < 0.04$). In addition, there was a significant effect of Time, $F(1, 11) = 7.65, p < 0.02$, with levels overall increasing from 30 to 180 min.

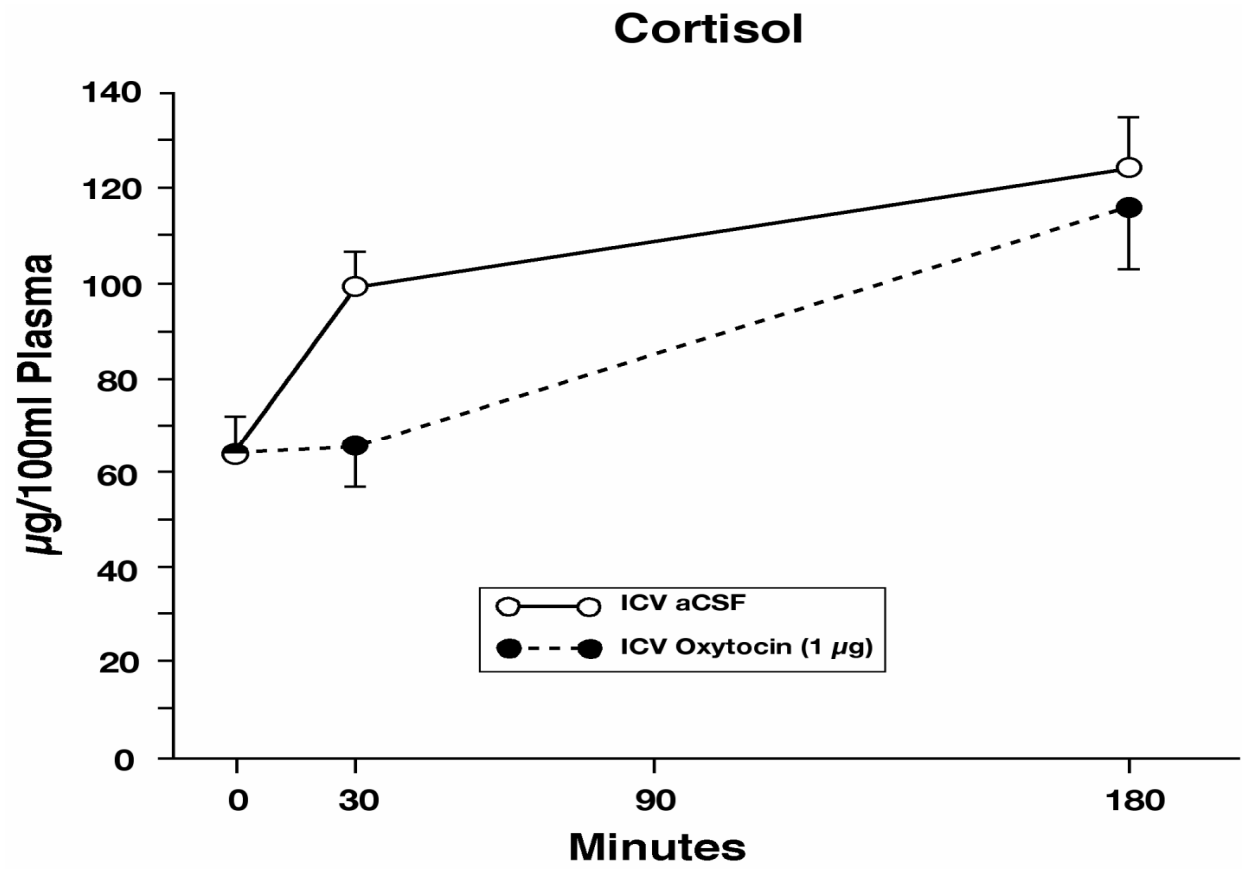


Fig.5. Mean basal plasma cortisol levels and levels at 30-min and 180-min following infusion of oxytocin or aCSF in Experiment 3

Active Behaviors. There was no significant difference in the number of vocalizations or line-crossings exhibited with the central administration of 1 μ g oxytocin vs aCSF in guinea pig pups ($N=12$). As in Experiments 1 and 2, the semi-interquartile ranges indicated that scores were very variable.

Table 3

Median and Semi-Interquartile Range (SIR) of Active Behaviors in Experiment 3

	Oxytocin(1µg)	aCSF
Vocalizations		
Median	33	41
SIR	88	467
Line-Crossings		
Median	14	7
SIR	30	92

Passive Behaviors. The Wilcoxon test revealed no significant effect for crouch, eye-close, piloerection, or the full passive response with the central administration of 1 μ g oxytocin vs aCSF in guinea pig pups ($N=12$). Still, the median values for all three individual passive behaviors following oxytocin administration are nearly half those following administration of aCSF.

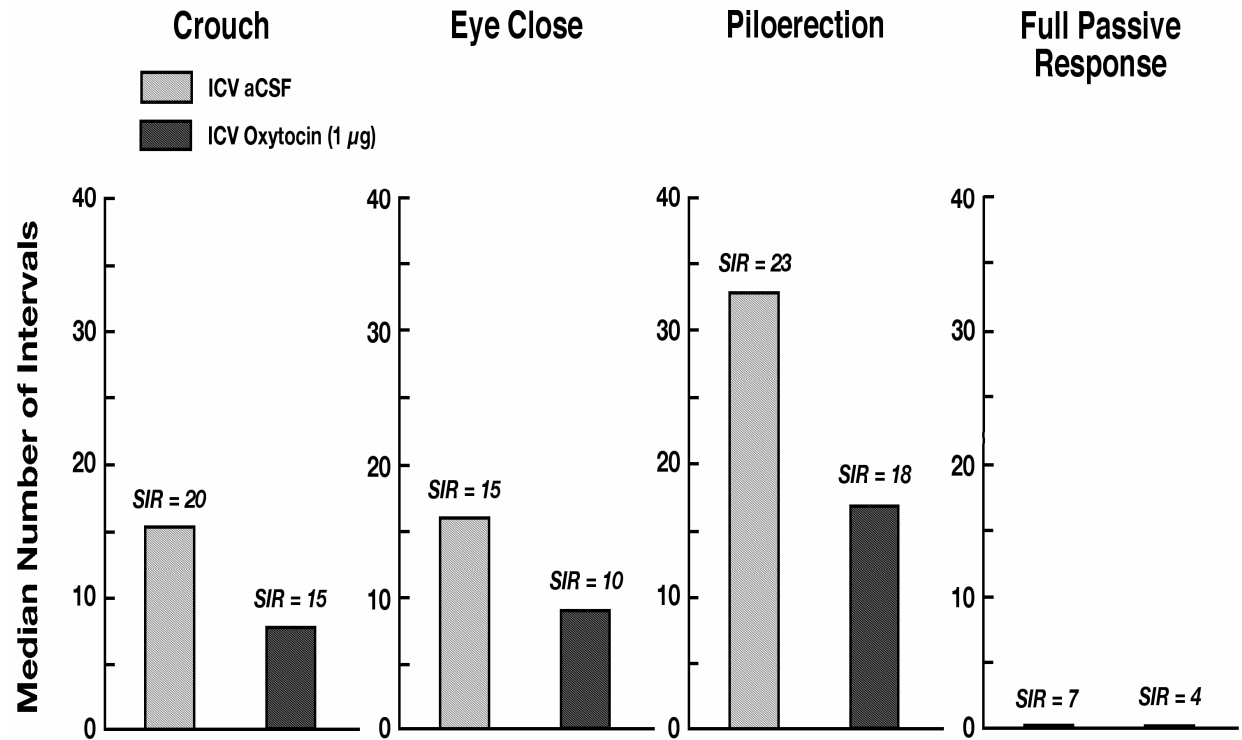


Fig.6. Median and semi-interquartile range (SIR) number of crouch, eye-close, piloerection, and the full passive response observed in female guinea pig pups during 180-min isolation test in Experiment 3

Discussion

A significant reduction in the circulating cortisol levels at 30-min following oxytocin administration was found, with levels overall increasing from 30-min to 180-min. The active behaviors were not significantly affected. No significant differences were found for crouch, eye-closure, piloerection, or the full passive response behaviors. However, the median values for all three passive behaviors were nearly half those with administration of oxytocin. This suggests a tendency for this dose of oxytocin to have a slight effect on reducing passive behaviors. This suggestion would need to be tested in further experiments.

General Discussion

The presence of the mother has been shown to reduce the activity of the HPA axis in her pups when exposed to a novel environment. Oxytocin is hypothesized to be released in the young of several species in the presence of their mother. At 180-min of separation in Experiment 1, oxytocin reduced plasma cortisol levels in isolated guinea pig pups. This mimics the effect that the presence of an attachment figure has and is consistent with the idea that oxytocin may be involved in the mother's ability to reduce HPA activity in her pups. However, the cortisol effect seen in the first experiment was not found in the second. The only differences between the two experiments were the addition of a second dose of oxytocin, 20 μ g, and the elimination of two blood samples. Because the 10 μ g dose did not produce a decline in cortisol levels as it had in Experiment 1, the added disturbance of the two additional blood samples in Experiment 1 may have contributed to the difference in findings between these two experiments. Oxytocin release is typically elicited with somatosensory stimulation. Because the pups in the first

experiment were handled more than those in the second, the added disturbance may actually have intensified the effect of oxytocin.

Another difference in the outcomes of Experiment 1 and 2 was that there was some reduction of passive responses with oxytocin in Experiment 1 but not Experiment 2. These passive responses are thought to be mediated by proinflammatory factors since administration of an anti-inflammatory has been shown to reduce these specific behaviors. Oxytocin is believed to have anti-inflammatory properties; a protective effect that may buffer the action of substances such as cytokines. However, data from this study suggests that oxytocin may only display anti-inflammatory action under certain conditions. In Experiment 1 only one passive behavior was significantly reduced with oxytocin administration and none were in Experiment 2. Yet, there was some tendency for the oxytocin condition to result in lower values of passive behaviors in both experiments.

Neither Experiment 1 nor 2 found significant effects on active behavior. The variability in these data may have contributed to the lack of significance. Oxytocin has been suggested to have anxiolytic-like effects in some animals; still, these effects may only be seen under certain testing conditions.

In Experiment 3, there was a significant reduction in the circulating cortisol levels at 30-min with oxytocin administration. The oxytocin was present in the CNS immediately upon infusion which may account for the effect being displayed after a short period of isolation. Central infusion of any substance ensures that the BBB has been traversed and the compound can act within the CNS. This suppression of the cortisol response was not present at 180-min.

No significant differences were found for crouch, eye-close, piloerection, or the full passive response with the central administration of 1 μ g oxytocin. However, the median values for all three individual passive behaviors were nearly half those with administration of oxytocin. This suggests a tendency for oxytocin to reduce the passive behaviors. Once again, there was no significant effect on the active behaviors.

Peripherally, oxytocin is best known for its hormonal actions during labor and lactation. Within the CNS its actions promote prosocial behaviors such as infant-mother attachment. The effects of centrally and peripherally administered oxytocin are different, but there is evidence that both play an important role in reducing the stress response. In this study, some aspects of oxytocin's capability to reduce responses to stress were revealed. The effect of central administration on behavior is more easily understood because the full dose of oxytocin is administered directly to the brain, whereas peripherally administered oxytocin must somehow exert action beyond the blood brain barrier. Both peripheral and central administration of oxytocin significantly reduced plasma cortisol levels at different time points of isolation. Oxytocin administered peripherally reduced the duration rather than the magnitude of the cortisol response. Data in this study suggest that oxytocin administered peripherally may take a longer time to influence the HPA axis. Peripheral administration of oxytocin also significantly reduced eye closure, a passive behavior; still, with both routes of administration oxytocin displayed a tendency toward the reduction of passive behavior.

The half-life of oxytocin is minutes; small doses degrade more quickly than larger doses. One μ g is a typical dose used for central infusion in rodents; yet larger doses may produce more significant results. Future studies might examine the effect of a larger

dose on active and passive behaviors. In addition, future studies should examine the route by which peripherally administered oxytocin produces its effects. Action on plasma cortisol levels could be exerted either centrally or peripherally at the level of the adrenal. How peripheral injection of the peptide might affect behavior is less clear. In summary, the present three experiments offer some, though limited, support for the hypothesis.

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